METHOD DEVELOPMENT AND VALIDATION GEMCITABINE AND CARBOPLATIN IN BULK AND IN ITS PHARMACEUTICAL DOSAGE FORMS USING HPLC AS PER ICH GUIDELINES

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Abstract

A new method was established for simultaneous estimation of Gemcitabine and Carboplatin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Gemcitabine and Carboplatin by using Inertsil ODS C18 5µm (4.6 x 250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M, pH 4.6) and Acetonitrile in the ratio 55:45%v/v and pH was adjusted with Orthophosphoric acid, detection wave length was 274 nm. The analytical method was validated according to ICH guidelines. The linearity study for Gemcitabine and Carboplatin was found in concentration range of 1µg-5µg and100µg-500µg and correlation coefficient was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 0.2 and 0.4, % RSD for intermediate precision was 0.5 and 0.1 respectively.

KEYWORDS: Gemcitabine, Carboplatin, Inertsil ODS and RP-HPLC.

Introduction

Gemcitabine hydrochloride diflurocytidine monohydrochloride has antitumour activity; the cytotoxic effect of Gemcitabine is attributed to combination of two actions of the diphosphateand the triphosphate nucleosides which leads to inhibition of DNA synthesis. In combination with cisplatin, it is useful as first-line drug for the treatment of adenocarcinoma of pancreas and also used as second line therapy in patients previously treated with fluorouracil. It is used alone or in combination with cisplatin for the treatment of advanced or metastatic bladder cancer. Carboplatin is an anticancer drug. Carboplatin is used to treat ovarian cancer. Carboplatin is also used for other types of cancer, including lung, head and neck, endometrial, esophageal, bladder, breast, and cervical; central nervous system or germ cell tumors, osteogenic sarcoma and as preparation for a stem cell or bone marrow transplant. An attempt was made to develop a straightforward, accurate, and affordable analytical method for the estimation of diphenhydramine and naproxen in light of the need for a suitable RP-HPLC method for routine analysis of simultaneous estimation of Gemcitabine and Carboplatin in pure and pharmaceutical Tablet dosage form. As per ICH criteria, the proposed approach will be validated.

Materials and Methods

Chemicals and Reagents: Pharmaceutically pure sample of Gemcitabine and Carboplatin was obtained from drug Manufacture Company, water, methanol, acetonitrile, potassium dihydrogen were of analytical grade.

Instruments: System (Waters 2690**),** Pump (Analytical HPLC isocratic pump, gradient pump)**,** Detector (waters 996 diode array detector), Software (empower 2 software)**,** Column (Kromosil (250×4.6mm, 5µ) ODS C-18 column)**,** Injector (Rheodyne injector with 20µ capacity)**,** Electronic balance (SHIMADZU electronic balance), Sonicator (Analytical Technologies Limited- Ultrasonic cleaner).

Chromatographic Conditions: The chromatographic conditions were successfully developed for the separation of Gemcitabine and Carboplatin by using Inertsil ODS C18 column (5µm, 4.6 x 250mm), flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The analytical method was validated according to ICH

guidelines (ICH, Q2 (R1)). The linearity study for Gemcitabine and Carboplatin was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was0.2 and 0.4, % RSD for intermediate precision was 0.5 and 0.1 respectively.

Preparation of Mobile Phase: 13.8g of sodium phosphate was accurately weighed and transferred in to 1000 ml volumetric flask, 2.5ml of phosphoric acid was added and 300 ml water was added. The solute was made to dissolve. Then the volume was made up to 1000 ml with water, pH was adjusted to 3.0. The solution was filtered through a 0.45 µm membrane filter. Phosphate buffer (pH 3.0) and acetonitrile was mixed in the ratio of 85:15, v/v. Then the solution was degassed with a helium spurge for 20 min.

Preparation of Sample solution: Stock solution of Gemcitabine and Carboplatin was prepared by transferring an accurately weighed quantity of 50mg of drug in to a 50 ml volumetric flask containing 20 ml of diluent, sonicated for 15 min and made up to the volume with diluent. Working standard of 100µg/ml was prepared from the stock solution by suitable dilution.

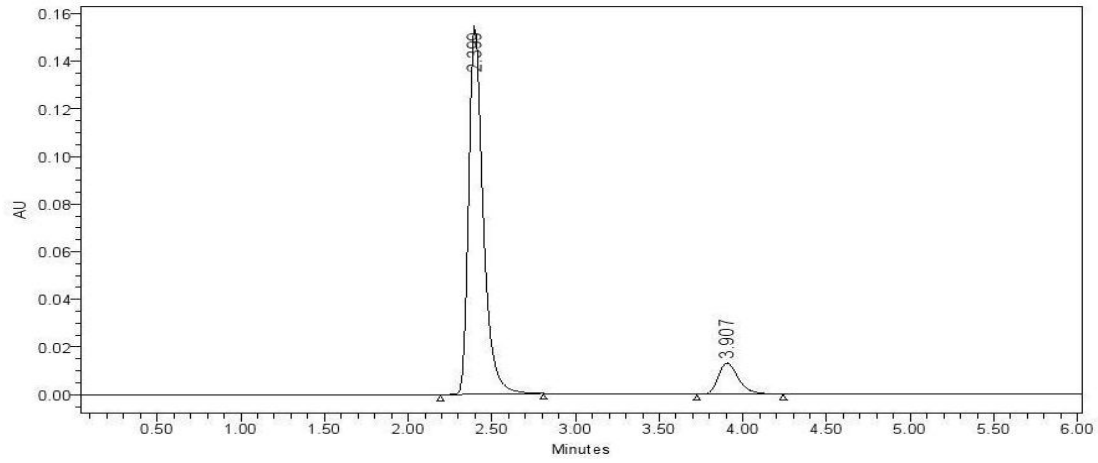

Fig: 1 Chromatogram of Gemcitabine and Carboplatin

S.No	Peak name	R_t	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Carboplatin	2.399	946124	155429	5105	1.3	8.1
2	Gemcitabine	3.907	111541	13239	3788	1.4	

Results

Validation Report

Linearity

The linearity study was performed for the concentration of 10 ppm to 50 for Gemcitabine and10ppm to 50ppm for Carboplatin.

Table: 1 Linearity results for Gemcitabine

S.No	Linearity Level	Concentration	Area
1.	I	10 ppm	83926
2.	II	20 ppm	1067774
3.	III	30 ppm	1246474
4.	IV	40 ppm	1439994
5.	V	50 ppm	1639065
Correlation Coefficient			0.99932

Table: 2 Linearity results for Carboplatin

S.No	Linearity Level	Concentration	Area
1.	I	10 ppm	626221
2.	II	20 ppm	778750
3.	III	30 ppm	931447
4.	IV	40 ppm	1070162
5.	V	50 ppm	1196060
Correlation Coefficient			0.99916

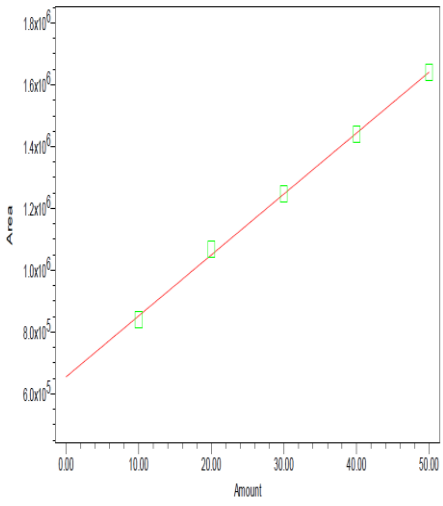

Fig: 2. Calibration curve of Gemcitabine

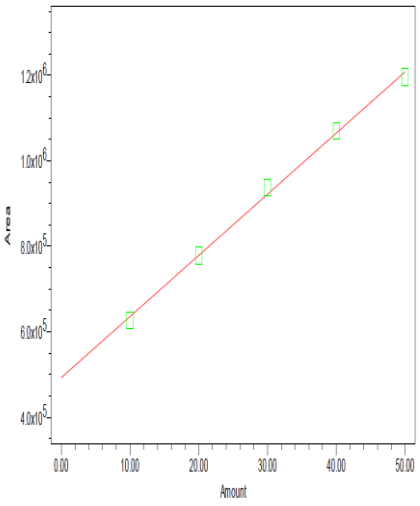

Fig: 3 Calibration curve of Carboplatin

Accuracy

The accuracy study was performed for 50%, 100% and 150 % for Gemcitabine and Carboplatin.The percentage % retrieval was found to be 98.0% and 102.0%.

Table 3: Chromatogram Values for Accuracy of Gemcitabine

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.13%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	15	14.89	99.2%	99.69%
		15	14.86	99.0%	
		15	14.82	99.79%	

Table 4: Chromatogram Values for Accuracy of Carboplatin

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.31%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	15	14.89	99.2%	99.89%
		15	14.86	99.0%	
		15	14.99	99.79%	

Precision (Repeatability)

The precision evaluation was performed for five injections of Gemcitabine and Carboplatin. Each standard injection was injected into chromatographic system. The intermediate precision study was performed for five injections.

Table 5: Intermediate Precision values for Gemcitabine

S.No	Name	RT	Area	Height (µv)
1	Gemcitabine	2.465	752386	111226
2	Gemcitabine	2.472	752118	112497
3	Gemcitabine	2.467	755566	110347
4	Gemcitabine	2.466	757638	109792
5	Gemcitabine	2.472	757330	110661
Mean			755008	
Std.Dev.			2638.6	
% RSD			0.35	

Table 6: Intermediate Precision values for Carboplatin

S.No	Name	RT	Area	Height (µv)
1	Carboplatin	4.323	412252	50991
2	Carboplatin	4.343	408090	50664
3	Carboplatin	4.324	414361	50295
4	Carboplatin	4.323	414692	49813
5	Carboplatin	4.342	411255	49826
Mean			412130	
Std.Dev.			2676.0	
% RSD			0.65	

LOD and LOQ

The LOD was performed for Gemcitabine and Carboplatin was estimated to be 3.04 and 3.08 respectively. The LOQ was performed for Carboplatin and Gemcitabine was estimated to be 9.79and 10.37.

Table 7: LOD and LOQ values for Gemcitabine and Carboplatin

S.No	Name	RetentionTime(min)	Area	Height(µv)
1	Gemcitabine	2.456	754122	112157
2	Carboplatin	4.312	419548	51017

Robustness

The robustness was performed for the flow rate variations from 0.4ml/min to 0.6ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Carboplatin and Gemcitabine which can be resulted that the variation in flow rate affected the method significantly.

Table 8: Robustness results for Gemcitabine (flow rate):

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	3041	1.7
2	1	3452	1.7
3	1.2	3107	1.7

Table 9: Robustness results for Carboplatin (flow rate):

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	6383	1.4
2	1	6353	1.4
3	1.2	6231	1. 4

Table: 10 Robustness results for Gemcitabine (Organic composition)

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	3463	1.7
2	Actual	3452	1.7
3	5 % more	2795	1.6

Table: 11 Robustness results for Carboplatin (Organic composition)

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	8488	1.3
2	Actual	4556	1.4
3	5 % more	4931	1.5

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Conclusion

The newly created RP-HPLC method is rapid, accurate, exact, and specific for the measurement of diphenhydramine and naproxen in tablet dosage forms. The suggested approach can be conveniently used for standard quality control analysis.

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